PROTHALLIA OF LYCOPODIUM IN AMERICA

II. L. LUCIDULUM AND L. OBSCURUM VAR. DENDROIDEUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 300

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(WITH PLATES XVI–XVIII)

In the first paper (4) there was included a short description of a single prothallium of *L. lucidulum;* also reference to what was thought at that time to be the prothallium of *L. obscurum* var. *dendroideum.* The question raised then was later settled in a short announcement published about a year later (5). It is the purpose of the present paper to describe fully the hitherto undescribed prothallia of these two species of *Lycopodium*, and to draw such comparisons as may seem justifiable.

Material and methods

The method of collecting described in the first paper has been followed almost entirely. It was found neither desirable nor practicable to sift or to wash the soil. The smallest desirable prothallia may be secured by the simple means of picking with curved forceps. A patch of soil in the form of a square or rectangle was removed to a depth of 4 or 5 cm., placed in a basket, and carried to the laboratory, where the prothallia were picked out. At frequent intervals small portions were placed under a dissecting microscope for the detection of the very youngest stages. Lying prone upon the ground is quite as good a method, save for the fact that one gets extremely tired after four or five hours of painstaking search.

For fixing, various mixtures of chromic-acetic-osmic acids were used, as well as formalin-alcohol. While the former proved to be somewhat better than the latter, it was not determined what is the best mixture.

The prothallia of *L. lucidulum* are ideal for a study of sex organs, since they are long dorsiventral plants with the organs mixed and growing acropetally from the apex. The main stages of both organs have been secured in a single section. This is rather rare, of course, but a comparatively few sections give one surprisingly good results. Since they can be secured very easily in the field because of their abundance, no other prothallia of *Lycopodium* offer so many advantages at once for class demonstration and laboratory material as these.

Lycopodium lucidulum

Since the prothallium of this species of *Lycopodium* was not known previous to my original brief description, it will not be out of place to give a rather detailed account of it.

Locality

Out of a total of more than 500 prothallia of *L. lucidulum* which were found, only about half a dozen were gathered outside the small region just north by several hundred yards of the Ridge Street Cemetery at Marquette, Michigan. Evidence of the existence of them was found at Mid-Island Point, Sugar Loaf Peak, Negaunee, Munising, Ishpeming, and Michigamme. There is no doubt that any region of the upper peninsula of Michigan will yield prothallia, providing, of course, that it is in general a habitat for the species. It is only because the present investigations were confined to a careful examination of a very limited region that a larger range cannot be given here. This limitation was made primarily to determine the relative abundance of material.

Occurrence

This species of *Lycopodium* produces prothallia in remarkable abundance. It is only necessary to become acquainted with the habitat and to exercise a due amount of patience and vigilance, to obtain a gratifying supply. All the prothallia were found within an area not more than 25 m. square. Within this area there were three especially rich patches, although these were not all discovered the same season. One was found in May, another in September, and the third in June of the following year. One patch measured

19 cm.×16 cm. and yielded 153 prothallia; another, measuring 18 cm.×14 cm., yielded 134 prothallia. The third patch was the most remarkable, being only 3 cm. square, and containing 74 prothallia. In the two larger patches there were 178 sporelings in all, 70 of which bore prothallia. Within the same general area in which the three rich patches of prothallia were found, were five other patches of the same general size, but which produced only sporelings. No effort was made to count them, but they were more numerous than the prothallia themselves, and were so closely crowded that many of them failed to produce more than the first set of leaves. In searching it was found to be much more profitable to seek patches of abundant sporelings than to wander about looking for isolated groups of them, even though these patches often failed to produce any prothallia in spite of the great number of sporelings. From this abundance of sporelings it is evident that not infrequently prothallia occur in even greater quantities than were actually secured from the richest patch found.

Relationship to spore-bearing plants

This investigation confirms the experiences of others who have found prothallia of *Lycopodium*, namely, that they occur most frequently where adult sporophytes are scarce. It seems probable that this situation must be explained by some difference in the soil. Since we know nothing of the conditions which govern the germination and development of *Lycopodium* spores, so far as the soil is concerned, a certain answer to the question is impossible. I have observed, however, that the soil in which prothallia grow is drier than that in which the adult sporophytes are found. This is especially true for the species under consideration. It is certain that an enormous number of spores must find their way to the soil beneath the cone-bearing plants, but the latter grow so thickly that sporelings would have little chance of survival, granting that prothallia had succeeded in growing. The sickly condition of many of the thickly growing sporelings found supports this idea.

As a matter of fact, prothallia do occasionally occur in even the densest growths of adult sporophytes, and it is not improbable that they might be found there in quantity. The fact that investigators consistently fail to uncover prothallia with adult sporophytes where the latter are growing thickly, in the opinion of the writer does not negative the probability, for it is most tedious to hunt the plants under these conditions. BRUCHMANN (1) has investigated old areas of sporophytes probably more carefully than any other investigator, but his method was first to remove the adult plants and then to look for sporelings. In doing this the majority of them would be destroyed. The writer has investigated the older plant areas, both by the method of BRUCHMANN and without first removing the adults. Four prothallia were found by the latter method and none by the former. Since the chief object was to secure prothallia, the waste of time necessary to secure only a few specimens among adult sporophytes caused the writer to abandon such areas.

The question naturally arises as to how the adult plants of *L. lucidulum* reach the habitats which are more moist than that of the prothallia. Let us assume that a prothallium has succeeded in growing on the side of a knoll or a hill, and that at the bottom of this knoll or hill is a moister habitat favorable for the growth of adult sporophytes. When the sporeling breaks through the surface of the ground it faces the hazards of drought and too much sunlight. If these are simultaneous or of sufficient duration, the plant must surely die. The observations of the writer are that this is a constant catastrophe in the struggle of these delicate plants. Occasionally, however, we may expect to find a plant growing under the conditions of a wet season. Then it progresses rapidly and becomes a hardy specimen. When it becomes an adult it is capable of producing spores and gemmae. It seems likely that it is the gemmae that play the chief rôle in further distribution, for when small clusters of these adult plants are found they almost invariably bear numerous gemmae, but rarely sporangia. When the gemmae fall in habitats favorable for the growth of prothallia, they may germinate well, and even form normal sporelings, but it is a significant fact that they appear unhealthy and generally show the incipient stages of extinction. On the other hand, those that reach a moister region, like that at the bottom of our assumed knoll or hill, grow vigorously.

The line of distributive succession is not easily shown in *L. lucidulum*, but a case of *L. obscurum* was found that well illustrates the idea. A sporeling of this species was found that was 10 cm. long, bearing six successive aerial branches and a distinct foot at the end of the underground trailing stem. The sporeling grew on the top of a small knoll, but instead of staying there, it took the shortest path toward the bottom, where adult sporophytes were growing in a moist hollow.

The rôle of prothallia in the life history of *L. lucidulum* is of slight value to the species, therefore, once a colony is firmly established. But for the opening up of new localities, and therefore for the geographical distribution of the species, the gametophyte plays the important part of getting a foothold in regions too unfavorable for vegetative parts, especially gemmae, to flourish. The sporeling itself rarely reaches maturity.

Two facts were observed in the collection of prothallia of various species which seemed to indicate that some agent other than wind takes part in the distribution of spores. The first of these was that the prothallia, in nearly all species, occur in bunches. The second was the frequent occurrence of prothallia in groups of four. The latter condition is explained by supposing that the original group of tetraspores was distributed bodily, and that the four germinated simultaneously. This phenomenon was observed very frequently in *L. clavatum*, *L. complanatum*, and *L. lucidulum*, most frequently in the first.

At the time of the shedding of spores the tetrasporic group is broken up, so that it does not seem probable that these groups are distributed by the wind. The only other explanation which suggests itself is that portions of the plant bearing sporangia are carried away by animals. The appearance of patches of prothallia and sporelings strongly suggests this. An isolated sporeling or gametophyte is rarely found.

HABITAT

Many searches for prothallia have been futile, not so much through a paucity of material as through a failure to learn, by a process of elimination, the exact type of terrain in which they grow most abundantly. It is difficult to describe what this is. In general one must follow the same principles involved while searching for the prothallia of any pteridophyte group.

For the prothallia of *L. lucidulum*, moisture is without doubt the most important factor. Countless millions of spores shed annually from an acre of these plants fail to produce prothallia, either because they are washed away into streams, driven too deeply into the soil, lodged in water logged depressions, or are subjected to dry conditions before and after germination. It is only when they fall upon a region whose sandy substratum is covered by a thin layer of leafmold, and is sufficiently protected from the seasonal drought and from inundation, that they germinate and produce prothallia. Such a place is a hillside or a hilltop with scattered trees, very little shrubbery, and a scattered representation of such herbs as the squawberry, wild sarsaparilla, *Clintonia borealis*, and *Polygala*. Very often grasses and *Polytrichum* are found in such a habitat. A sandy knoll with sparse vegetation and with a swamp at its base is an excellent locality.

BRUCHMANN found many of his prothallia of *L. Selago*, a related species, in mats of moss. The writer found very few in such places. The rich patches just described lay on a sandy hillside and were protected only by the leaves which fell the previous autumn. The soil was a rich humus for a depth of 1 cm., with almost pure sand beneath. Although hundreds of sporelings were found, many were in a desiccated condition, and only a very few would ever have become adults, for adult plants habitually occur in regions a little more moist than where the prothallia and sporelings were found.

Because the prothallia of this species of *Lycopodium* were found growing in such a variety of places, the following list of these may be valuable: (1) in a hole at the base of a living birch tree; (2) among the decayed needles of *Pinus resinosa;* (3) under a hard maple tree; (4) on the top of a rotten stump; (5) on partly shaded hillsides; (6) in the sand under a patch of *Polytrichum* and *Polygala;* (7) in sand scarcely covered at all with humus; (8) in moldy humus (most abundantly); (9) in muck, at the edge of a permanent pond; (10) in the wheel tracks of an abandoned forest road.

It might be well to add that the habitat of the prothallia of this species of *Lycopodium* is somewhat more shaded, more moist, and more protected from sunlight than that for *L. clavatum*, *L. complanatum*, and *L. annotinum*. The prothallia of five species have been found within a few centimeters of each other, however. They are most abundant at a depth of 1 cm., and seldom grow at a depth of more than 2 cm. Very frequently they occur upon the surface of the ground. The habitat of these prothallia agrees in all essential respects with that for *L. Selago*, with the exception already mentioned, which seems to indicate that they require a slightly drier place for growth than do those of the latter.

Description

The older regions of the prothallia are brownish, while the young growing tips as well as the very young prothallia are white. It is these very white tips that are first noticeable when digging in the soil. They are closely beset with multicellular hairs (fig. 49), which in some cases include as many as five cells. They are evidently of a glandular nature, for in fresh specimens of prothallia the region which bears them is covered with a thin mucilaginous secretion slightly denser than water. If the prothallia are placed in water, this substance quickly swells and is dissolved. Whatever its nature, no doubt it plays the important rôle of keeping the young growing region and the young sex organs from becoming dried. None of the fungus filaments which closely adhere to the exterior of other parts of the prothallium ever pierce it, nor do soil particles or leaf mold adhere to it. It is probable that the growing apical region is lubricated sufficiently by this substance to prevent injury during its upward passage through the soil and mold, which are often very compact and solid.

The adult prothallia vary very much in size and shape (cf. figs. 2–41). These figures are all drawn to a scale of four, so that they are just twice the size of the original specimens.

The form of the prothallium does not conform exactly to that of any type heretofore described. It approaches nearest that of *L. Selago* described by Bruchmann. Taking fig. 15 as the type form, it will be noticed that there is a lower cup-shaped primary region, from which has developed a more or less dorsiventral protuberance which becomes cylindrical near its apex only. In no case were there found the two forms of body as described for *L. Selago*, namely, the cylindrical or "ninepin" form and the flattened form. All the specimens of the vertically growing prothallia of *L. lucidulum* might roughly be considered as shaped like a "ninepin" or an Indian club, but upon close examination it has been found that sex organs grow only on one side, the rhizoids opposite and lateral to them, and that the symmetry is distinctly dorsiventral. It is only when active growth is taking place that the apical region ever becomes cylindrical at all. Fig. 51 shows a longitudinal section through the apex of a prothallium such as that shown in figs. 15 or 49. This bilateral symmetry is present from the very beginning of the differentiation from the meristem tissue.

If the prothallia grow upon the surface, however, they are distinctly of the *L. clavatum* or *L. annotinum* type (figs. 33, 35). Even when growth continues after a subterranean prothallium has reached the surface, this growth produces an expanded portion which rests upon the ground (figs. 34, 37, 39). In other words, when the length of the vertical portion is eliminated so that the primary tubercle lies immediately beneath the prothallium, we have a type in no essential respect different from that of *L. annotinum.* Figs. 32 and 37 show prothallia semisurface and semisubterranean in development. In many instances (figs. 25, 28) horizontal growth is solely below the surface, and it is not until near maturity that the apical point turns abruptly vertical. In such instances the prothallia are evidently of the *L. annotinum* type originally, and later change.

In short, there are three methods of growth: one in which growth is entirely vertical; one in which growth is first vertical, then horizontal; and another in which growth is first horizontal and then vertical. In several instances growth was originally vertical, later assumed the horizontal, and still later resumed the vertical.

While a prothallium of *L. Selago* has never been examined, I cannot see from the figures of BRUCHMANN any evidence of a typically "conical" or "ninepin" shape for that species of prothallium. His fig. 32, plate VI, is the nearest approach to it, but the form is far from being typical, as the drawings to the left of it show. Even in fig. 32 the sex organs occur on one side, while the vegetative organs are mainly opposite and lateral to it. Apparently the only difference between the form of the prothallia of the two species in question is the alleged conical shape of *L. Selago* prothallia, and I am not able to see that BRUCHMANN'S figures admit even of this difference. If I were to use the same method of drawing as he has, it would be almost impossible to distinguish between the typical forms of prothallia of the two species. BRUCHMANN'S figs. 22, 28, and 29 are especially easy to match with prothallia of *L. lucidulum*. My figures merely accentuate the different regions of the prothallia, while his suggest them.

For the present, therefore, the prothallium of *L. lucidulum* is placed with BRUCHMANN'S type for *L. Selago*, but it is probable that the necessity for such a type is one of convenience rather than a morphological one. For the sake of reducing the growing number of types of prothallia for the genus *Lycopodium*, it might be well to include these representatives of the *L. Selago* type in the *L. clavatum* type.

Chlorophyll occurs regularly and abundantly in the subepidermal cells of all prothallia of *L. lucidulum* which were found growing upon the surface. Four prothallia were found which had bifurcated. The age of the prothallia varied greatly, as the figures show. There seems to be some evidence that the prothallia of *L. lucidulum* have a much shorter period of development than BRUCHMANN found for his European species. Solely from field observations I should estimate that adult prothallia for *L. lucidulum* probably mature within two or three seasons, but the period for *L. obscurum* must be much longer.

TISSUES

A longitudinal section of a prothallium like that shown in fig. 50 shows two main regions, an upper and a lower. The upper region is divided into a distinct epidermis, which bears the sex organs and paraphyses, and a subepidermal mass of cells of parenchymatous structure which contain chlorophyll (when this is present) in its upper half and reserve starch in the lower half. A cross-section is shown in fig. 64. This set of tissue, therefore, is reproductive and assimilative in function, in its upper portion, while its lower portion is employed for the storage of food material. The starch grains are compound. While they occur most abundantly in the lower half of the upper set of tissues, very frequently they are found also in the cells immediately below the epidermis, in the venter of the archegonium and the cells of the fungus regions (fig. 64). Approximately the lower half of the prothallium is inhabited by the endophytic fungus. This will be described later.

The meristematic tissue (figs. 51, 52) lies at the apical end of the prothallium in a depression between the two groups of tissues just named. A single apical cell is probably not present. Growth is apparently brought about by periclinal and anticlinal division of a small number of cells which form a meristematic plate between the youngest portions of the upper and lower sets of tissues. The mitotic figures of fig. 51 show that division continues in cells which lie at a considerable depth. The lobed-like mass of tissue which bends downward over the apical region and which bears the primordia and older cells of the sex organs is caused by this division of the interior cells, as well as by the rapid division of the more superficial ones. The cells of the meristematic plate, from which are cut off immediately the vegetative and reproductive primordia, are filled with dense protoplasm and numerous oil drops (fig. 53).

Rhizoids are very abundant, and are simple elongations of epidermal cells on the lower side. No case was observed in which a rhizoid was cut off by a cross wall.

Sex organs

The prothallia are monoecious. The sex organs appear in acropetal succession (fig. 51), the older ones being located (fig. 50) in the tissue lying above the primary tubercle. Both antheridia (figs. 54–58, 62) and archegonia (figs. 42–47) develop by the usual stages known for the genus. The largest number of canal cells found was four. A double row of these was not observed. The ventral canal cell is very much flattened at maturity. The egg is slightly oval at maturity, with the long axis perpendicular to the canal. It almost completely fills the venter. The neck of the archegonium is short, and the venter lies completely below the level of the epidermis. The neck is not provided with a cover cell. It opens by the dissolution of the inner walls of the four terminal cells which border each other at a common line. As soon as fertilization takes place further development of archegonia normally ceases. Consequently two embryos on the same prothallium are rarely found, and since fertilization occurs after the opening of the canal, the embryo therefore lies very near the meristematic region. The growth of the embryo causes activity there to stop, and as a result it is found as a rule at the end of the prothallium.

Antheridia appear earlier and more abundantly than archegonia, and are scattered throughout the entire upper surface of the prothallium. They are circular or oval in outline, and may be submerged entirely or may form a slight elevation on the upper part of the prothallium. They are isolated and never form antheridial masses similar to those very characteristic of *L. obscurum* to be described later. Figs. 58 and 59 show the latter stages in the development of the sperm.

The sex organ primordia lie so close together that it is impossible always to say what kind of organ will develop. They may be separated by a single layer of cells, or, as Miss LYON (3) observed in *L. annotinum*, they may touch one another. They are evidently of such a primitive nature that it is impossible to distinguish an antheridium from an archegonium in the very earliest stages of their development. There are some indications that the archegonial initial is slightly larger and longer than the antheridial initial, but this is so uncertain that it is useless as a criterion.

As a result of this primitive condition, mixed sex organs are very frequently found. These may be normally shaped antheridia in which a few cells have never divided into sperm mother cells, or they may be archegonial in form, the neck filled with normal spermatogenous tissue, and the ventral canal and egg cells of female appearance (fig. 60). The opposite condition may also occur (fig. 61). Figs. 60 and 61 are sketches of two archegonia constructed from camera drawings of nine sections in serial order. Six abnormal organs of a bisexual nature were found on one prothallium. They of course always occur near the apex of the prothallium. The fact that sexual organs of a bisexual nature occur on plants where primordia of sex organs are apparently alike in position, suggests that we may be dealing with one of the primitive stages of sex organ development. HOLFERTY (2) found the same indifferent situation in *Mnium cuspidatum*. Miss LYON brought together considerable information on several genera of pteridophytes regarding the condition in abnormal archegonia. A longitudinal section is best for the study of sex organs.

Only the first division in the development of the prothallia from the spore was observed. Fig. 1 illustrates the single specimen which was found in field material.

Lycopodium obscurum dendroideum

The prothallia of this species were most difficult to find. The sporelings have an appearance so much like those of *L. complanatum* that a certain abnormally growing prothallium of the latter species was described in my first paper (4) as that of *L. obscurum*. This error was corrected as soon as discovered (5). A total of thirty-seven prothallia were found. Twelve were dug up in a patch 2 feet square near the locality described for *L. lucidulum*, and very near to a juniper bush. The others were found near Mid-Island Point, Michigan. The exact spot lies just behind a row of cottages, about 300 yards from Lake Superior and in an alder clump between the lake and the marsh to the east of it. This spot was visited first in July and later in August of 1917. It was very dry at this time, but is probably water-logged for at least two months of the year. Twenty-five prothallia of *L. obscurum*, fifteen of *L. complanatum*, and six of an undetermined variety of *L. clavatum* were found in an area not more than 10 feet square.

The soil in the first locality contained very little humus in the sand. A species of grass and *Polytrichum* grew about the spot, which was fully exposed to the sun, and no leaf mold was present. The sand was yellowish a short distance below the surface, and rather compact. The spot rests on a ridge about 15 feet higher than the surrounding land. The lake bed once covered this point, and it is probably an old sand bar.

The soil of the second locality could hardly offer a more contrasting condition as to physical appearance. The subsoil of course was sand, but this was covered by a soil mixture of half leaf mold and half muck, covering lake sand. As a matter of fact, some of it was nearly pure mycelial threads. Other parts were as hard as dry muck itself. The prothallia found here grew for the most part in the intensely moldy soil. Some were found at the border between the humus layer and the sand lying beneath it. From this it will be seen that one cannot name a type of soil as a criterion.

If it is desirable to indicate the type of place to look for *Lycopodium* prothallia, I should say that one should look in neither the dry nor the wet places. For my own part I search in those spots a little less moist than the habitat of the adults. In the case of *L. obscurum* and *L. clavatum*, very often the reverse is true, but just as one cannot give a precise type of soil and moisture content for the sporophytes, so he cannot for the gametophytes.

Sporelings were found in a variety of other places near Marquette and Munising. The prothallia of *L. obscurum* probably grow abundantly wherever the sporophyte has been known to exist for a long time and in considerable quantity. Two prothallia and one sporeling were found in July 1922, near Rhinelander, Wisconsin. A sporeling and an old prothallium of *L. complanatum* were found in June 1922, near Pembine, Wisconsin.

Description of prothallia

The prothallia are of the *L. annotinum* type and not of the *L. complanatum* type suggested in my first paper (4). They are about as smooth as the *L. annotinum* prothallia, and never as wrinkled or lobed as those of *L. clavatum*. While the first are yellowish brown and the second dark brown, those of *L. obscurum* are reddish brown. They are also somewhat larger than those of the other species. The largest one found measured 8×12 mm.; the smallest was about the size of a pin head. Figs. 65 to 71 show them just twice the natural size. Owing to the fact that the rhizoids are so few that the soil scarcely clings to them, they may be distinguished by this means also. The young of the three species just mentioned and *L. complanatum* cannot always be distinguished, but identity of species can generally be determined by the habit of growth of the endophytic fungus. Confusion of species is sometimes unavoidable, however, and this is especially true when prothallia of several kinds and of young age are found in the same lump of soil. The writer discovered such a mistake after a four months' study of a single slide. Certain features relative to the fungus were found on it that did not occur in any of the other specimens to which it purported to belong. In fact, it was this discrepancy that led to the discovery of the mistaken identity of the prothallia under discussion. The habit of growth of the endophytic fungus will be discussed later.

Fig. 70 shows a specimen of *L. obscurum* which looks like *L. complanatum.* The color and endophytic fungus both place it with the species assigned. Furthermore, the remarkable habit of growth of the antheridia was found present here, although the drawing does not show it. The enlarged antheridial mass shown in fig. 81 was taken from a specimen of a large *L. complanatum* prothallium. Both were rotted away at the lower end. Now no published figure of the last named species of prothallium shows that antheridia grow in such enormous masses. These facts can mean only one of two things, namely, that the fact of antheridial mass growth has not been observed, or that the form of *L. obscurum* prothallium is really intermediate between the *L. complanatum* and *L. annotinum* types. In view of the fact that other observers of *L. complanatum* prothallia were unlikely to overlook so conspicuous a feature, I am inclined to the view that the prothallia of *L. obscurum* occasionally take the intermediate position between the two well known types. After careful examination of the specimens found there is small likelihood of mistaken identity. If this be true, what I have called a prothallium of *L. obscurum* in my first paper (4) may need no modification. Moreover, the prothallia of *L. Selago*, *L. lucidulum*, and the peculiar specimen of *L. obscurum* all show a lateral groove more or less extensive, and are dorsiventral.

Sex organs

The antheridia grow in enormous masses of white beadlike knobs. These may half encircle the entire prothallium in the adult, or may cause the very young to resemble cornucopiae. Such structures have not been observed by the writer on any other prothallia with certainty. Certain prothallia previously figured (4) seem to suggest them, but at that time prothallia of *L. obscurum* were unknown, and identification was made upon comparison with figures published by other writers. It is entirely possible that those specimens may have been misnamed. There is also the possibility that antheridia may occasionally take the form mentioned in species other than the one under discussion. Figs. 79–81 show the habit of growth of these organs. The prothallia are all shown under a magnification of ten, so that a glance at the figures will give the relative size of the individuals figured. Fig. 81 shows a mass enlarged.

A cross-section of the prothallium through two antheridial ridges is shown in fig. 74. It is apparent that the masses arise from the tissue lying within the outer border ring. For sake of clearness, one region is represented as without the endophytic fungus. As a matter of fact the fungus does invade the antheridia through the opening shown by the opercular cells in figs. 82 and 89. It has not been located for certain in the cells of the antheridia walls, but it is possible that the fungus is present in the intercellular spaces. Because of the intimate relationship between the sperms, and possibly the wall cells, with the fungus, it seems very likely that the unusual growth of antheridial masses may be explained by symbiosis. There is no indication of a pathological condition of the sperms. The writer has never seen the fungus in the antheridia of any other species of prothallia of *Lycopodium*. It is well known that an endophytic fungus present in the prothallia of certain ferns will cause abnormal growth of tissue.

The individual antheridia arise acropetally from the groove. There is no primordium for the mass. This is a secondary growth, although it arises very early. Figs. 87 and 88 show the antheridium initial and the first division. The mature detail is shown in figs. 82 and 89. In this species four opercular cells may often be observed. I have never seen it for certain in any other species. The figures representing these cells were made by camera lucida.

In all of the prothallia sectioned, numerous retarded antheridia were observed. They were distributed throughout the older regions of the plant, and had the ordinary appearance of the male organs, but the mother cell walls were extremely weak and the nuclei small. They appeared starved. The endophytic fungus was not demonstrated to be present in them, and it is very probable that they originated in the usual way.

Mitosis does not occur simultaneously throughout the individual antheridia. The organ is divided into quarters, and all the cells of a single quarter will show the same phase, but one earlier or later than the neighboring quarter.

ARCHEGONIA

The stages in the development of individual archegonia are the same as those described for the genus. A few of the stages are shown in figs. 83–86. The neck is rather long, containing as many as fourteen canal cells. As a rule these are double, or at least part of them are. Even the ventral cell is involved in this division (fig. 86). In this organ the cells are beginning to disintegrate, as shown by the swollen walls. The position of such an organ relative to the prothallium is shown in fig. 90 on the cut edge. That the doubling of the neck canal cells occurs early is shown by fig. 85. One instance was observed of a very abnormal archegonial growth. Six organs arose from the prothallium (fig. 90), which were all apparently normal, near maturity, and about the same age. There were traces of the endophytic fungus in the canals. The fungus occurs normally in all the archegonia. It is probable that this peculiar massing of archegonia is to be explained on the same grounds as the antheridial masses. It would be instructive to know how frequently this occurs. Unfortunately the mass was not discovered until sections had been made, and the topographic view had to be made up from serial sections.

The exact morphological position of the sex organs, especially the antheridial masses, should be made plain. From a superficial view of fig. 80, it might appear that the upper mass is derived from the border, but the section shown in fig. 74 should make the matter clear. It was rather difficult to determine the origin of the mass. Antheridia of all ages are found in any one, but the primordia always are found at the outer junction of the mass and the prothallial tissue beneath. Nevertheless, certain sections showed that the primordia were slightly raised above the surface, as though dragged there by rapid growth of the surrounding tissue. While I am satisfied that there is no definite primordium for the mass, I am not at all certain that the mass itself may not begin development before the antheridial primordia appear; at least convincing evidence was not found. If the fungus is responsible for the growth, almost any order may be expected in the development of some individual organs. It was not possible to be certain of the relationship between the developing mass and the developing antheridia. The organs are so numerous that only one of two things can occur, either the whole group must spread itself, or most of the individuals must be absorbed. There is conclusive evidence in the figures that few are absorbed.

Endophytic fungus

The study of the endophytic fungus has delayed the publication of this paper considerably. The following account refers to that found in the two species of prothallia under discussion. Plate oo has been added to show some of the structures observed. The identity of the fungus has not been established. It does not appear to be the same in both species. Both the reproductive structures and the habit of growth support this statement. Furthermore, there is evidence that they may both be Ascomycetes.

The illustrations are arranged so that the fungus of *L. obscurum* is represented in the upper half of the plate (figs. 91–110), and that of *L. lucidulum* in the lower half (figs. 111–126). Each group is subdivided into reproductive and vegetative structures. No attempt has been made to insure the morphological identity of any structure represented. In the *L. obscurum* group, figs. 94–105 are supposedly reproductive structures, and figs. 106–110 vegetative. In the *L. lucidulum* group, figs. 113–117 and figs. 122–126 are supposedly reproductive, while figs. 118–121 are vegetative. The structures shown in figs. 124 and 125 were found in both types of prothallia. They are all shown under the same magnification, and are collected from over 500 drawings.

All the reproductive structures, except in figs. 124–126, were intracellular. The vegetative structures were intercellular. The ascomycetous growth of fig. 124 was very common, but always was found just outside the epidermis. It was found connected with the intracellular mycelium, however. The mass shown in fig. 125 was often observed in decayed regions of the prothallia. Since the "spores" shown in fig. 126 were found in such a mass, it is probable that the fungus sometimes destroys the prothallium in the older parts, to distribute its own spores. At any rate, many prothallia of *L. complanatum* were found with the entire lower half rotted away, and all the prothallia of *L. obscurum* sectioned, and a few of *L. lucidulum* had rotten holes in them which were filled with the fungus.

Habit of growth

The fungus enters the prothallium either through the rhizoids or between the epidermal cells. To say that it enters is not exactly accurate. It may be possible to show that many of the "entering" mycelia are in reality leaving the prothallium. It undoubtedly gets a foothold during the earliest life of the plant. To within two or three cells of the meristematic cells it is established (figs. 50, 51), which shows that it is eminently capable of taking care of itself, once it has obtained a foothold.

In *L. lucidulum* the fungus occupies the region shown in fig. 111, but there is one peculiar thing to be noted. Only about half of the cells contain the mycelial threads, the other half contain bodies which in some respects resemble spores (fig. 112). These show no nuclei under various stains. With safranin and aniline blue the walls reacted like cellulose. In a few cases minute red chromatin-like granules appeared differentiated, but these were never seen after iron alum haematoxylin; consequently, it is impossible to state just what these bodies actually are. In a few instances very minute mycelial threads were seen to radiate from pores in the walls of these bodies, as though they were germinating spores. These were always seen in section and cannot alone be considered conclusive evidence that they are indeed such structures. So far as this investigation goes, it cannot be said that they do or do not have any relation to the endophytic fungus. They are such a constant structure associated with the fungus, however, that some connection seems extremely probable. One other fact should be noted. The obviously reproductive structures, found abundantly in the cells which lie next to those containing the sporelike bodies, are found without these sporelike bodies in the region neighboring the meristematic tissue. In other words, the sporelike bodies are not found in the cells which abut the meristematic cells. Since the structures which looked like germinating spores and appeared to contain chromatin also appeared in those very cells where the typical sporelike bodies were wanting, the evidence is still stronger for the spore interpretation. A few microchemical tests were made to determine the exact nature of the cell wall of these bodies, but nothing definite was established.

In *L. obscurum* the habit of growth of the fungus is markedly different. The reproductive bodies are larger, and there are none of the small sporelike bodies so persistent in *L. lucidulum.* All the cells of the infected region contain the mycelium, but it is much less extensive than in *L. lucidulum* (figs. 91, 93). Here there are two lower cells with a very regular and tight coil of the mycelium, which is much denser than shown in the illustration, where clearness was desired. Above this layer is a region of finer coils, generally about one cell in thickness. The coils change their orientation slightly in the next layer above, until those of the uppermost layer are at right angles to those of the lowest layers. The reason for this is probably one of absorption, for the mycelial threads finely divide and closely abut the walls of the palisade tissue which lies above and which contains reserve starch. These fine threads were never observed to enter the palisade cells, nor did their tips show swelling. This very evident difference in habit constitutes my first reason for believing that the fungus in the two species of prothallia under discussion is not the same. The second reason involves the reproductive-like structures. A glance at these structures at the top and bottom of the plate will reveal organs drawn to the same scale, but vastly different in detail. The only possible comparison may be found in figs. 97 and 125. The latter may be only a later stage of the former. The lefthand cell of fig. 112 when compared with fig. 97 shows the contrast well. The organs of the last named figure occur only in the lower cells of fig. 93, but those of fig. 112 are equally distributed over the fungus region of *L. lucidulum.*

In spite of these observations, there is another which needs to be noted, and which may cast doubt upon them. The structures shown in fig. 124 were found on the epidermis of both kinds of prothallia. That shown in fig. 125 was never found except in the decayed portions of both kinds of prothallia. I suspect that the latter is only the maturer stage of the former. The "spores" of fig. 126 were drawn from a ruptured heavy walled structure shown in the lower lefthand corner of fig. 125. There were sixteen spores. All these facts point to the ascomycetous nature of the fungus, but since these last named structures were all found without the epidermis or within decayed regions where external fungi would have access, it is conceivable that they have no relation whatever to the endophytic fungus. The internal mycelium was so constantly segmented, however, that the Oomycete group has little chance of being recognized.

While the evidence presented is in no respect conclusive, it throws some doubt upon the *Pythium* theory as to the identity of the endophytic fungus of *Lycopodium* prothallia. The writer dislikes very much to leave so important a question unsettled, but it seemed best at least to record what had been observed.

Summary

1. The prothallia of two more species of *Lycopodium* have been discovered.

2. The sex organs of *L. lucidulum* are primitive, being frequently mixed in nature.

3. The prothallium of *L. obscurum* var. *dendroideum* shows a form transitional between the *L. annotinum* and *L. complanatum* types.

4. The sex organs of the last named species are invaded by the endophytic fungus and consequently show deformities. The antheridia and rarely the archegonia occur in enormous masses.

5. The sex organs develop individually as described for the genus, and acropetally in the two species.

6. The endophytic fungus is probably not *Pythium*. The reproductive structures point to this genus, but the vegetative and certain doubtful structures point to the Ascomycetes.

7. The habit of growth, and the appearance of the reproductive structures, indicate that the same species of fungus is not present in the two prothallia.

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EXPLANATION OF PLATES XVI–XVIII

PLATE XVI. PROTHALLIA OF L. lucidulum

FIG. 1.—Germinating spore, with first wall transverse; found in field.

FIGS. 2–41.—Habit sketches of various typical prothallia, some of which bear sporelings attached; ×2.

FIGS. 42–48.—Stages in development of archegonium; ×375.

FIG. 49.—Tip of prothallium, showing five antheridia and multicellular hairs.

FIG. 50.—Longitudinal section of prothallium.

FIG. 51.—Apex of same, showing acropetal succession of sex organs; meristem plate of cells lies just behind first initial.

FIG. 52.—Detail of meristem and initial plate; ×375.

FIG. 53.—Single cell from meristem region; ×775.

FIGS. 54–57, fig. 62.—Stages in development of antheridium and sperms; ×375.

FIGS. 58, 59.—Stages in sperm development; ×775.

FIGS. 60, 61.—Mixed sex organs, reconstructed from serial camera drawings; ×375.

FIG. 63.—Longitudinal section of embryo; ×85.

FIG. 64.—Cross-section of prothallium, showing distribution of starch grains.

SPESSARD on LYCOPODIUM